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# *Environmental Effects of Dredging Technical Notes*



## **Critical Body Residue (CBR) Approach for Interpreting the Consequences of Bioaccumulation of Neutral Organic Contaminants**

### **Purpose**

This technical note describes a procedure for interpreting tissue residues of neutral organic chemicals generated in 28-day dredged material bioaccumulation bioassays. This interpretive guidance uses a critical body residue (CBR) of neutral organic chemicals reported for the fathead minnow, *Pimephales promelas*. The CBR is based on a very large U.S. Environmental Protection Agency (EPA) acute toxicity database and well accepted quantitative structure activity relationships (QSARs). Guidance in this technical note is not appropriate when xenobiotic metabolism of neutral organic contaminants is likely.

### **Background**

The evaluation of dredged material requires an assessment of "unacceptable adverse impacts." Testing to support this evaluation will often include sediment bioassays. One type of bioassay determines the bioaccumulation potential of sediment-associated contaminants. In this test, aquatic organisms are exposed to sediments for 10 or 28 days, depending on whether heavy metals or organic chemicals, respectively, are the contaminants of concern. Tissues of animals surviving the sediment exposure are chemically analyzed to evaluate bioaccumulation potential. Interpreting the biological importance of these bioaccumulation data (with regard to "unacceptable adverse impacts") has been problematic. Previous guidance to Corps field elements has been based on published peer-reviewed articles containing both contaminant tissue residues and the corresponding biological effects (see Bibliography). While this guidance is technically sound, its limited size and large test-to-test variations preclude broad generalizations.

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AQM00-11-3594

The relationships among acute toxicity, level of exposure, and internal chemical dose have been examined in the fathead minnow, *Pimephales promelas* (McCarty and others 1985, McCarty 1986 and 1990). For a wide variety of neutral organic chemicals, the estimated internal body burden corresponding to acutely lethal exposures was remarkably constant — 4.4 mmol/kg wet weight\* (95 percent confidence interval (C.I.) = 3.7 - 5.2 mmol/kg, n = 150) (McCarty and others 1992). This conservative internal dose is referred to as the critical body residue (CBR). The CBR is based on a very large database of 96-hr LC<sub>50</sub>s generated by the U.S. Environmental Protection Agency Environmental Research Laboratory-Duluth (Brooke, Geiger, and Northcott 1984, Geiger and others 1985 and 1986, and Geiger, Call, and Brooke 1988); the bioconcentration QSARs of Mackay (1982); and the toxicity QSAR approach of Konemann (1981) and Veith, Call, and Brooke (1983). This technical note describes how the CBR reported for *P. promelas* can be used to interpret the biological consequences of bioaccumulation in dredged material bioassays.

## Additional Information

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## Approach

Using the CBR to interpret 28-day bioaccumulation data for neutral organic chemicals is simple and straightforward. The four-step procedure is described below and summarized in Table 1.

### Step 1

The first step is to express the original bioaccumulation data for each neutral organic chemical as milligram per kilogram wet weight. All contaminants must be considered even if the level of bioaccumulation was not statistically significant. If the original data are reported on a dry weight basis, multiply the concentration by (1.00 minus the proportion body water) to obtain wet weight-specific data. If percent body water of the test species is not known, 80 percent is a reasonable approximation (Lagler, Bardoch, and Miller 1962, Florey 1966, Emerson 1969, and Tucker and Harrison 1974). If concentrations are reported on a lipid basis, multiply by (1.00 minus the proportion of lipid) to

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\* In the original publications, tissue concentrations were reported as both mmol/kg and mmol/L. All residues are reported as mmol/kg in this technical note, which assumes an organism density of approximately 1.0.

**Table 1. Summary of Procedure for Using the Critical Body Residue (CBR) of Neutral Organic Contaminants in *P. Promelas* to Interpret Results of 28-day Bioaccumulation Bioassays**

Step 1	Express bioaccumulation data for all neutral organic chemicals as milligram per kilogram wet weight
Step 2	Convert the milligram per kilogram wet weight tissue concentrations from step 1 to millimoles per kilogram wet weight
Step 3	Multiply the millimoles per kilogram wet weight concentrations from step 2 by appropriate acute-to-chronic ratios to produce an estimated acute tissue concentration (EATC) for each neutral organic chemical
Step 4	Add up all EATCs from step 3. Compare this sum with the CBR for <i>Pimephales promelas</i> (4.4 mmol/kg). One of the following conclusions will emerge: If the sum of the EATCs is greater than CRB, "unacceptable adverse impacts" are likely. If the sum of the EATCs is less than CRB, "unacceptable adverse impacts" are unlikely.

obtain a weight-specific concentration. If percent lipid was based on a dry weight sample, convert to wet weight concentrations as above.

### **Step 2**

The second step involves converting each milligram per kilogram wet weight tissue concentration obtained in step 1 to millimoles per kilogram wet weight. To accomplish this, simply divide the molecular weight of each contaminant into its milligram per kilogram wet weight tissue concentration. One millimole of any chemical is equal to its molecular weight expressed in milligrams. Table 2 gives the atomic and molecular weights of many common elements and contaminants of concern. Additional atomic and molecular weights can be found in most chemistry textbooks or Verschueren (1983). If the molecular weight of the chemical of concern is not readily available, simply add up the atomic weights of all atoms in the molecule. The sum total of atomic weights is equal to the molecular weight.

### **Step 3**

Because bioaccumulation data generated in chronic (28-day) exposures are to be compared to a CBR which is estimated from acute (96-hr) exposures, some basis for normalizing this time difference is needed. One normalizing factor is the acute-to-chronic ratios published by the EPA (Table 3). The acute-to-chronic ratio is obtained by dividing the exposure concentration associated with chronic toxicity into the acutely lethal concentration; usually the 96-hr LC<sub>50</sub>. If no acute-to-chronic ratio has been calculated for the contaminant and test species of concern combination, a default value of 10 is recommended.

**Table 2. Frequently Used Atomic and Molecular Weights**

Atom	Atomic Weight*	Contaminant	Molecular Weight**
Aluminum	227	Acenaphthylene	152
Arsenic	74.921	Acenaphthene	154
Barium	137.34	Anthracene	178
Boron	10.81	Aroclor 1016	257
Bromine	79.904	Aroclor 1221	192
Calcium	40.08	Aroclor 1232	221
Carbon	12.011	Aroclor 1242	261
Chlorine	35.453	Aroclor 1248	288
Fluorine	18.998	Aroclor 1254	327
Hydrogen	1.008	Aroclor 1260	372
Iron	55.847	Benzene	78
Magnesium	24.305	Benzo(a)anthracene	228
Mercury	200.59	Benzo(a)pyrene	252
Nickel	58.70	Benzo(e)pyrene	252
Nitrogen	14.007	Biphenyl	154
Oxygen	15.999	Chlorobenzene	113
Phosphorus	30.974	Bieldrin	381
Potassium	39.102	DDD	320
Silicon	28.086	DDT	355
Silver	107.87	Fluoranthene	202
Sodium	22.990	Mirex	546
Sulfur	32.06	Naphthalene	128
Tin	118.69	Perylene	252
Zinc	65.37	Phenanthrene	178
		Phenol	65
		Pyrene	202
		Tetrachloromethane	154
		Toluene	92

\* From Morrison and Boyd (1973).

\*\* From Verschueren (1983) and Mackay, Shiu, and Ma (1992a, 1992b).

**Table 3. Acute-to-Chronic (A:C) Ratios Published in the EPA Water Quality Criteria Documents (U.S. Environmental Protection Agency 1980) for Freshwater and Marine Organisms**

Contaminant	Test Species	A:C Ratio
Chlordane	<i>Daphnia magna</i>	3.6
	<i>Cyprinodon variegatus</i>	20
	<i>Lepomis macrochirus</i>	37
Chlorine	<i>Daphnia magna</i>	5.227
	<i>Menidia peninsula</i>	1,162
	<i>Gammarus pseudolimnaeus</i>	>37.18
	<i>Pimephales promelas</i>	6.162
Dieldrin	<i>Mysidopsis bahia</i>	6.2
	<i>Salmo gairdneri</i>	11
	<i>Poecilla reticulata</i>	9.1
DDT and metabolites	<i>Pimephales promelas</i>	65
Endosulfan	<i>Daphnia magna</i>	11
	<i>Mysidopsis bahia</i>	2.8
	<i>Pimephales promelas</i>	3.0
	<i>Cyprinodon variegatus</i>	2.4
Endrin	<i>Palaemonetes pugio</i>	19
	<i>Pimephales promelas</i>	2.2
	<i>Cyprinodon variegatus</i>	1.9
	<i>Jordanella floridae</i>	3.3
1,2-Dichloroethane	<i>Pimephales promelas</i>	5.9
1,1,2-Trichloroethane	<i>Pimephales promelas</i>	8.7
1,1,2,2-Tetrachloroethane	<i>Pimephales promelas</i>	8.5
Pentachloroethane	<i>Mysidopsis bahia</i>	1.4
	<i>Pimephales promelas</i>	6.6
Hexachloroethane	<i>Pimephales promelas</i>	2.8
Butylbenzyl phthalate	<i>Daphnia magna</i>	42
	<i>Pimephales promelas</i>	15
Heptachlor	<i>Pimephales promelas</i>	80
	<i>Cyprinodon variegatus</i>	3.9
Hexachloro-cyclohexane (Lindane)	<i>Daphnia magna</i>	33
	<i>Chironomus tentans</i>	63
	<i>Pimephales promelas</i>	7.5
Naphthalene	<i>Daphnia magna</i>	11
PCBs	<i>Gammarus pseudolimnaeus</i>	11
	<i>Pimephales promelas</i>	6.4
Pentachlorophenol	<i>Daphnia magna</i>	2.5
	<i>Pimephales promelas</i>	3.9
	<i>Cyprinodon variegatus</i>	6.9
Toxaphene	<i>Daphnia magna</i>	109.1
	<i>Mysidopsis bahia</i>	1.132
	<i>Pimephales promelas</i>	196
	<i>Cyprinodon variegatus</i>	1.540
	<i>Ictalurus punctatus</i>	28

(Kenaga 1982 and Mayer, Mayer, and Ellersieck 1986). Once the appropriate acute-to-chronic ratio has been identified, multiply it by the chronic bioaccumulation tissue concentration (obtained in step 3) to yield an estimated acute tissue concentration (EATC) in millimoles per kilogram wet weight for each neutral organic chemical.

#### Step 4

Add up all the EATCs obtained in step 3 and compare this sum to the CBR for neutral organic chemicals in *P. promelas* (4.4 mmol/kg). One of the following conclusions will emerge.

- If the sum of EATCs is greater than CBR, "unacceptable adverse impacts" are likely.
- If the sum of EATCs is less than CBR, "unacceptable adverse impacts" are unlikely.

An example calculation using hypothetical tissue residue data from a 28-day dredged material bioaccumulation bioassay is shown in Table 4.

#### Analysis

The above procedure is based on a number of assumptions. A major assumption is the validity of the CBR itself. One argument in its favor is the presumed mode of toxicity — nonspecific narcosis. This is "the reversible state of arrested activity of protoplasmic structures" (Veith and Broderius 1990). Neutral organic chemicals partition into the lipid portion of biological membranes because they are hydrophobic. Their presence as dissolved constituents in the lipid phase is believed to swell the membrane beyond a critical volume (Seeman 1972 and Franks and Lieb 1985). This swelling disrupts cellular structure and function and results in the overt symptoms of narcosis—lethargy, unconsciousness and death in extreme narcosis. This type of toxicity is called nonspecific narcosis because it affects biological membranes in general, not specific tissues; it has been observed in a very wide variety of organisms (plants, mammals, fish, and invertebrates); it can be induced by any neutral organic chemical; and the effects are additive. This mode-of-action suggests that the internal contaminant dose, expressed on a molar basis (that is, equal number of molecules), would be relatively constant for a variety of chemicals. This is precisely what is observed for the CBR estimated for *Pimephales promelas*.

Support for the validity of the CBR also comes from empirically determined acutely lethal tissue concentrations in aquatic organisms. Because the CBR is an estimated value, it is appropriate to compare it with empirically derived data gathered under the same or similar conditions (that is, acute exposures). Although such data are limited, the summary provided by McCarty and others (1992) indicates that acutely lethal tissue concentrations measured in crustaceans, insects, and other fish species agree reasonably well (that is, within single-digit range) with the estimated CBR for *P. promelas* (4.4 mmol/kg).

**Table 4. Example Calculation of Procedure Summarized in Table 1; Hypothetical Wet Weight Concentrations are from 28-day Dredged Material Bioaccumulation Bioassay with the Deposit-Feeding Marine Bivalve,  
*Macoma nasuta***

Contaminant	Molecular Weight	Tissue Concentration		A:C*	EATC**
		mg/kg	mmol/kg		
Phenol	65	0.1	0.002	10	0.02
Benzene	78	0.3	0.004	10	0.04
Toluene	92	0.2	0.002	10	0.02
Naphthalene	128	0.9	0.007	10	0.07
Biphenyl	154	0.6	0.004	10	0.04
Acenaphthene	154	1.4	0.009	10	0.09
Phenanthrene	178	2.1	0.012	10	0.12
Anthracene	178	1.8	0.010	10	0.10
Benzo(a)anthracene	228	3.7	0.017	10	0.17
Benzo(a)pyrene	252	4.2	0.017	10	0.17
Pyrene	202	5.9	0.029	10	0.29
Perylene	252	2.6	0.010	10	0.10
Chlorobenzene	113	0.7	0.006	10	0.06
Tetrachloromethane	154	0.1	0.001	10	0.01
Aroclor 1254	327	3.2	0.010	10	0.10
DDD	320	1.4	0.004	10	0.04
Dieldrin	381	0.8	0.002	10	0.02
Mirex	546	0.2	0.000	10	0.00
Sum of EATCs =					1.46

Sum of EATCs (1.46) < CBR (4.4)

Therefore, "unacceptable adverse impacts" are unlikely.

\* Acute-to-chronic ratio.

\*\* Estimated acute toxicity concentration.

A critical assumption in the procedure described in this technical note is that the toxicity and bioaccumulation potential of the freshwater fish, *P. promelas*, is representative of aquatic species in general. With respect to toxicity, Suter and others (1987) demonstrated that *P. promelas* is an acceptable surrogate test species for other freshwater fish. The EPA recommendation to evaluate toxicity of dredged material elutriates with *P. promelas* suggests that the agency believes it is an acceptable representative species. Both bioaccumulation and dose-response toxicity were reported for *P. promelas* following chronic exposures to

PCB-contaminated sediment (Dillon 1988). The advantages of using *P. promelas* in freshwater sediment bioaccumulation bioassays were discussed by Mac and Schmitt (1992). They also described in detail the bioaccumulation test procedure currently used for this species.

The toxicity and bioaccumulation QSARs used to estimate the CBR for *P. promelas* (4.4 mmol/kg) are based, in part, on the partitioning behavior of neutral organic chemicals between aqueous and lipid phases. These QSARs essentially treat aquatic organisms as "bags of lipids." Thus, lipid normalization tends to minimize differences among species. The CBR for *P. promelas* assumed a lipid content of 5 percent. If more divergent but realistic values are used (for example 3 and 8 percent), the mean CBR (95 percent C.I.) varies only slightly and remains within single-digit range; 2.6 mmol/kg (2.2 to 3.1) and 7.0 mmol/kg (5.9 to 8.3), respectively (McCarty and others 1992). Thus, the uncertainty introduced by interspecific differences in percent lipid appears to be minor.

There are, however, a number of reasons for questioning whether the CBR for *P. promelas* is representative of aquatic organisms in general. Suter and Rosen (1988), for example, demonstrated that extrapolating toxicity test results from fish to crustaceans introduces unacceptably large amounts of error. They speculated this may be due to interspecific differences in xenobiotic metabolism. Fish have highly developed contaminant metabolic capabilities. Other phylogenetic groups, such as mollusks, have very limited abilities. This is a major reason why deposit-feeding bivalve mollusks are frequently used in saltwater sediment bioaccumulation bioassays. A functionally equivalent freshwater mollusk has not been identified. A bioaccumulation test with the oligochaete, *Lumbriculus variegatus*, has recently been proposed (Call and others 1992). However, the capacity of this organism to metabolize xenobiotics has not been critically examined with regard to sediment bioassays.

If the validity of the CBR is accepted, then the major source of uncertainty in the procedure described herein is the link between acute and chronic toxicity. Acute-to-chronic ratios published by the EPA are used to establish this link. The acute-to-chronic ratio is obtained by dividing the chemical concentration associated with chronic toxicity into the acutely toxic concentration, usually the 96-hr LC<sub>50</sub>. The chronic value is based on results observed in partial or full life-cycle toxicity tests with aquatic organisms. It is derived from the lowest concentration where adverse biological effects were observed (lowest observed effects concentration or LOEC), the highest concentration where no adverse effects were observed (highest no effect concentration or HNEC), or the geometric mean of the LOEC and HNEC. The exact derivation varies with each chemical and each chronic laboratory experiment. If an acute-to-chronic ratio is lacking, a default value of 10 is recommended (Kenaga 1982 and Mayer, Mayer, and Ellersieck 1986). This default value is believed to be environmentally conservative for most organic chemicals.

Although acute-to-chronic ratios are empirical observations, there are some fundamental mechanistic reasons why acute and chronic toxicity should not, or in some cases, cannot be linked. The mode of acute toxicity of neutral organic

chemicals is believed to be nonspecific narcosis (see discussion above). While this mode of action can also produce chronic toxicity, other "specific" mechanisms may be more important in some animals. One mode of action requires bioactivation of the contaminant molecule via xenobiotic metabolism. The classic example is biotransformation of benzo(a)pyrene to the more toxic diol epoxide. Since xenobiotic biotransformation to toxic metabolites is not an important consideration in acute toxicity, the link between acute and chronic toxicity is lost if the species of concern has significant xenobiotic metabolizing capability. Another "specific" mechanism inducing chronic, but not acute, toxicity is associated with coplanar molecules such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and certain polychlorinated biphenyl (PCB) congeners. This receptor site-mediated mode of action does not require bioactivation by the xenobiotic metabolism system. For these reasons, it is recommended that the procedure outlined in this technical note not be used with isosteres of TCDD or if the test species has a well developed xenobiotic metabolizing system. In either case, the link between acute and chronic toxicity would be tenuous.

The conclusion reached in step 4 regarding the probability of "unacceptable adverse impacts" can never be a stand-alone criterion. That is, the decision regarding the acceptability of dredged material cannot be based solely on the results observed in step 4. Rather, it represents only one of many inputs to the technical evaluation of dredged material. Other considerations include the magnitude of bioaccumulation relative to the reference, the proportion of contaminants accumulated, sediment toxicity, volumes of material involved as well as potential management alternatives. The procedure in this technical note is simply an additional tool for evaluating the consequences of bioaccumulation in aquatic organisms.

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